

MicroXANES Characterization of Temporal Changes in the Zinc Environment of Zebrafish (*Danio rerio*) Embryos

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Introduction

Zinc is critical to the growth, development and differentiation of all species¹. Thus, for example, zinc deficiencies during development are often teratogenic, with effects ranging from mild developmental retardation to embryo death, depending on the severity of the deficiency². Despite its obvious importance, the molecular-level function of zinc in embryonic development is poorly understood. There are hundreds of zinc proteins, each with a different Zn site, and each likely to be present in variable amounts as a function of developmental stage. Precise definition of Zn distribution is therefore a daunting task.

Embryos that develop without maternal nutrition must possess all of the nutrients required for normal development at the time of fertilization. Such “closed” systems make excellent models to study the migration and speciation of zinc during development. Recently, Vallee and colleagues working with *Xenopus laevis* (African Clawed Toad) oocytes³ and Berg and colleagues working with *Danio rerio* (Zebrafish) embryos⁴ have reported that a large amount of zinc (~1mM) is stored within the embryos at the time of fertilization. Subsequent studies using ⁶⁵Zn were performed on the *X. laevis* embryos to follow the distribution of zinc during embryonic development⁵. The results of these experiments showed that the zinc remained associated with the lipovitellin protein in yolk platelets until the organism reached the tadpole stage of development (~48 hours after fertilization). The interpretation of these results was that zinc did not play a role in the early stages of development but was stored in the lipoproteins until a much later stage of development, at which time it was distributed to other zinc metalloproteins as needed.

Due to the relatively large concentration of zinc in the embryos, such samples are directly amenable to study via x-ray absorption spectroscopy (XAS). XANES (x-ray absorption near edge structure) spectra measured on intact embryos of various stages during early development for both zebrafish and *X. laevis* demonstrated that significant changes occurred in the bulk zinc environment within the first hour after fertilization, and that the zinc environment remained dynamic throughout the first 12 hours. These results were in contrast to the previous studies on *X. laevis* made using ⁶⁵Zn labeled vitellogenin.

Results

Taking advantage of the small spot size and high brightness of beamline 10.3.2, zinc microXANES spectra have been measured in the yolk and blastomere regions of the zebrafish embryos at different stages of development. Figure 1 shows the XANES and difference spectra from the yolk of the 4-cell, 128-cell and high stage embryos, while figure 3 contains XANES and difference spectra from the blastomeres of the 4-cell and high stage embryos. Despite the high noise level in these spectra, it is apparent that the yolk XANES in the latter stages of development are different from the yolk XANES of the 1-cell embryo. Comparing the trends observed in the embryo yolk XANES with

those of zinc model complexes⁶, the persistent positive difference at ~9663 eV and negative difference at ~9670 eV suggests that there is an increase in low-Z (N/O) ligand zinc sites found in the yolk at the later stages of development. The XANES spectra measured in the blastomere regions of the 4-cell and high stage embryos also show some differences. However, when difference spectra are calculated by subtracting these blastomere XANES from the XANES spectrum for the 1-cell yolk, the differences appear to be quite similar. The negative difference at ~9663 eV and the positive difference ~9670 eV suggests that the zinc sites in the blastomere have a larger number of sulfur ligands than the sites found in the yolk of the 1-cell embryo. Future efforts will focus on improving the signal to noise ratios in these spectra, so that direct comparisons may be made with spectra of crystallographically characterized zinc model complexes.

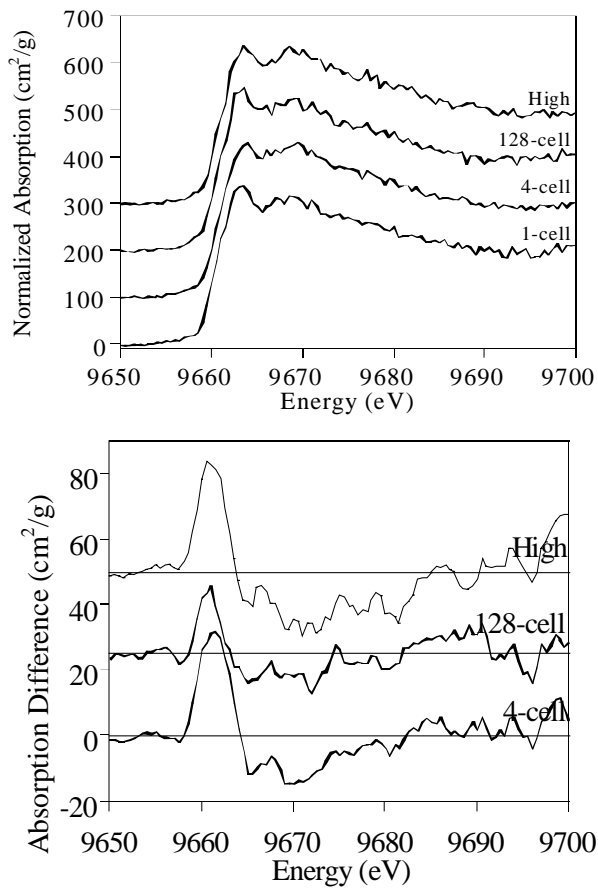


Figure 1: Zinc XANES (top) and difference spectra (bottom) measured with a 4 μm^2 beam positioned in the yolk of fertilized zebrafish embryos. These spectra show that changes occur in the zinc environment of the yolk within the first hour of development.

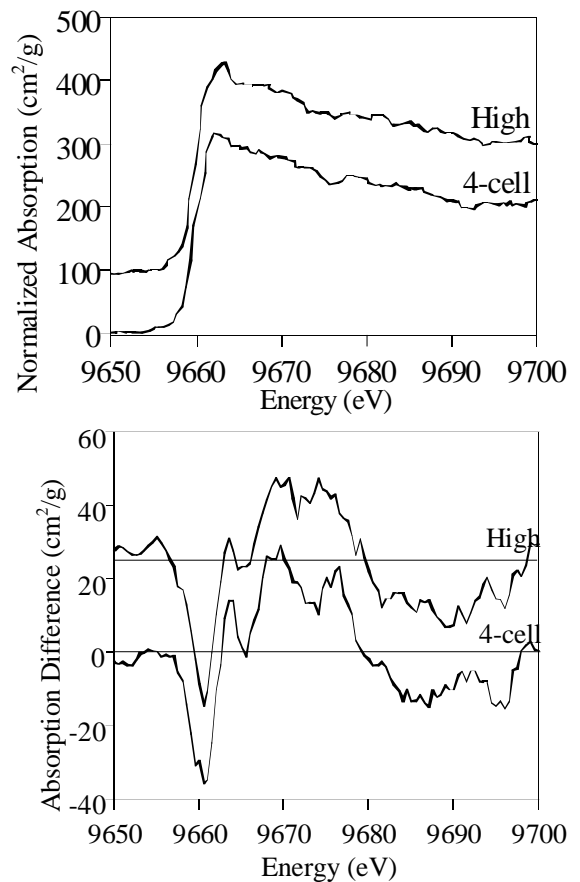


Figure 2: Zinc XANES (top) and difference spectra (bottom) measured with a 4 μm^2 beam positioned in the blastomeres of fertilized zebrafish embryos. The differences observed in the blastomeres are opposite those observed in the yolk, suggesting that there are distinctly different zinc environments localized in the yolk and blastomeres of the embryos.

Given that the 1-cell yolk is not only different from the yolk of the later stage embryos but also from the blastomeres, it can be ascertained that the changes observed in

the intact embryo XANES were due to changes in the zinc sites within both the yolk and the blastomeres. Since the changes observed in the yolk manifested themselves opposite of those from the blastomeres, it can also be inferred that the zinc sites in the blastomeres are dominated by sulfur ligands, while the zinc in the yolk tends toward a larger percentage of low-Z (N/O) ligands.

Since the microspectroscopy line is equipped to measure fluorescence signals from more than one element simultaneously, it has also been possible to measure Fe K α maps for the zebrafish embryos. The elemental maps suggest that the iron is highly localized within the blastomeres. The quantity of iron was sufficient to measure microXANES spectra in the blastomere region of the embryo. Figure 3 shows the preliminary Fe microXANES spectra from the 4-cell and 128-cell embryos. While this data demonstrates that it will be possible to deduce the oxidation state and coordination environment from such XANES measurements, future measurements will need to be signal averaged for a significant amount of time given that the iron concentration in the embryo is less than 500 μ M.

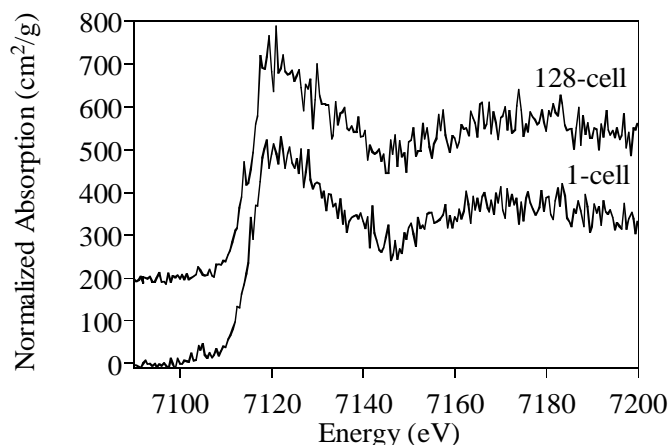


Figure 3: Fe XANES spectra measured in the blastomeres of newly fertilized (1-cell) and two-hour old (128-cell) zebrafish embryos.

References

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This work was supported by a grant from the NIH (GM-38047).

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